

# PROCEEDINGS OF SPIE

[SPIDigitalLibrary.org/conference-proceedings-of-spie](http://SPIDigitalLibrary.org/conference-proceedings-of-spie)

## Mouse brain imaging using photoacoustic computed tomography

Yang Lou, Jun Xia, Lihong V. Wang

Yang Lou, Jun Xia, Lihong V. Wang, "Mouse brain imaging using photoacoustic computed tomography," Proc. SPIE 8943, Photons Plus Ultrasound: Imaging and Sensing 2014, 894340 (3 March 2014); doi: 10.1117/12.2039669

**SPIE.**

Event: SPIE BiOS, 2014, San Francisco, California, United States

# Mouse brain imaging using photoacoustic computed tomography

Yang Lou, Jun Xia, and Lihong V. Wang

Department of Biomedical Engineering, Washington University in St. Louis,  
St. Louis, MO 63130

## ABSTRACT

Photoacoustic computed tomography (PACT) provides structural and functional information when used in small animal brain imaging. Acoustic distortion caused by bone structures largely limits the deep brain image quality. In our work, we present *ex vivo* PACT images of freshly excised mouse brain, intending that can serve as a gold standard for future PACT *in vivo* studies on small animal brain imaging. Our results show that structures such as the striatum, hippocampus, ventricles, and cerebellum can be clearly differentiated. An artery feature called the Circle of Willis, located at the bottom of the brain, can also be seen. These results indicate that if acoustic distortion can be accurately accounted for, PACT should be able to image the entire mouse brain with rich structural information.

**Keywords:** Photoacoustic computed tomography, small animal imaging, mouse brain structure, *ex vivo* imaging

## 1. INTRODUCTION

In small animal research, imaging physiological features and molecular changes can help to diagnose brain diseases such as brain cancer or trauma. Non-invasive and non-ionizing methods can also benefit treatments for brain cancer<sup>[1]</sup>. Neuroactivities in the brain are closely related to the functions of specific brain regions, such as the ventricular system, the hippocampus region or the colliculus. Therefore, images showing structural changes or bio-chemical composition of small animal brain are highly desired in neuroscience research, making small animal brain imaging a crucial role in the fields of biology, biomedical engineering, and medicine.

Photoacoustic imaging is a novel and powerful imaging modality that utilizes optical excitation and acoustic detection to provide both functional and molecular information about the brain. Previous literatures show that this technique can be applied in mouse/rat brain imaging for various purposes<sup>[2–7]</sup>. Wang *et al.*<sup>[2]</sup> demonstrated that photoacoustic tomography (PAT) can image mouse brain structures at different depths; in another report, Wang *et al.*<sup>[4]</sup> showed that PAT imaging of the rat brain can reveal functional information about brain activity; still other reports demonstrate that extrinsic contrast agents can also be injected to enhance contrasts<sup>[5;6]</sup>. However, a common challenge shared by previous studies is poor imaging quality at the deep brain level. The central cortex vessel has been the major physiological marker for PAT imaging. It typically indicates an imaging depth of around 1 to 2 mm for a mouse. Deeper structures can hardly be seen by PAT with a satisfying resolution.

Our work in this report focuses on answering the following question: how well and how deeply can PACT image without acoustic interferences. We did *ex vivo* imaging of excised mouse brains without the surrounding bone structures or soft tissues. In this case, the image quality should be much better than previous *in vivo* data. Our experimental results indicate that *ex vivo* imaging can reveal almost all the major structures at a depth of 3 mm; moreover, the penetration depth of PACT can cover the entire mouse brain, identifying features even at its bottom. These *ex vivo* experiments is to provide a reference for future *ex vivo* and *in vivo* mouse brain studies. This report first describes the experimental procedure and system setup, and then gives the results of *ex vivo* experiments, followed by discussion of experimental issues and conclusions.

---

(Send correspondence to Lihong V. Wang)

Lihong V. Wang: E-mail: lhwang@seas.wustl.edu

## 2. SAMPLE PREPARATION AND SYSTEM SETUP

To perform *ex vivo* mouse brain imaging, mouse brain sample was properly prepared. First, the mouse was euthanized in a closed environment using carbon dioxide, as specified by the Animal Control Protocol of Washington University in St. Louis. Then the ears were cut off, and the scalp was removed using surgical scissors. Brain excision followed the steps in Spijker<sup>[8]</sup>. Because blood is the contrast agent for PACT imaging, it should be preserved in the brain as much as possible during this process. The captivation mentioned in Spijker<sup>[8]</sup> causes a loss of blood from the major vessel, so we did not include captivation in our experiment (more discussion is forthcoming in 4). After the brain was excised, it's put into 10% Gelatin solution in refrigerator for preservation. The time lapse between excising and imaging is approximately 1 hour.

A detailed description of the photoacoustic computed tomography system can be found in Gamelin *et al.*<sup>[9;10]</sup>, and a schematic is shown in figure 2. The brain sample was put on a motor-driven base that can move vertically, letting the fixed transducer array scan different depths of the sample. The sample was situated in the center of the transducer ring to gain better imaging quality. Laser light (at wavelengths ranging from 535 to 975 nm) perpendicularly illuminated the sample. To receive photoacoustic signals, we used a full ring transducer array, with 512 elements, a diameter of 10 cm, and a center frequency of 5 MHz.

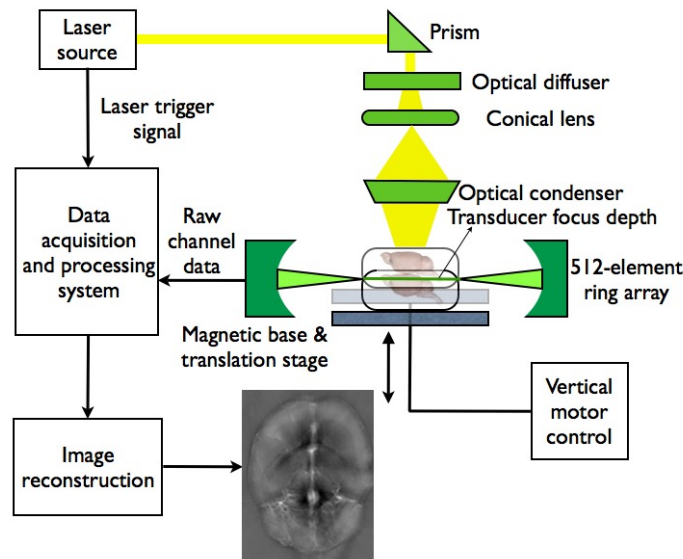


Figure 1. Photoacoustic computed tomography system setup

The laser sources in our setup were a Symphotic Ti-sapphire LT2211A (for near infrared wavelengths) and a Newport OPO Basiscan (for visible wavelengths). We used four wavelengths— 532 nm, 570 nm, 600 nm, 650 nm— on the OPO laser for all the samples and used three wavelengths— 750 nm, 800 nm and 975 nm— on the Ti-sapphire laser for some of the samples. The range covers from visible light to infrared. The choice of the wavelengths was based on the absorption spectrum of oxy- and deoxyhemoglobin.

WE used a three-dimensional back-projection based reconstruction algorithm proposed by Xia *et al.*<sup>[11]</sup>. To gain more details in the reconstructed image, we also explored additional methods: deconvolving the transducer's impulse response to cancel out the frequency response of the transducer and filtering with a Chebyshev high pass filter to enhance image details.

### 3. EXPERIMENTAL RESULTS

We did a number of *ex vivo* experiments to differentiate brain structures at different depths. Each image is paired with an experiment ID to indicate the date of data acquisition.

#### 3.1 3 mm depth

Figure 3.1 shows a PACT image of the *ex vivo* mouse brain (experiment #113012) at around 4.4 mm below the bregma, which is a landmark on the skull. The thickness of the skull is around 1 mm, so the actual depth is around 3 mm. Figure 3.1 allows comparison between our PACT image and a standard mouse brain atlas<sup>[12]</sup>.

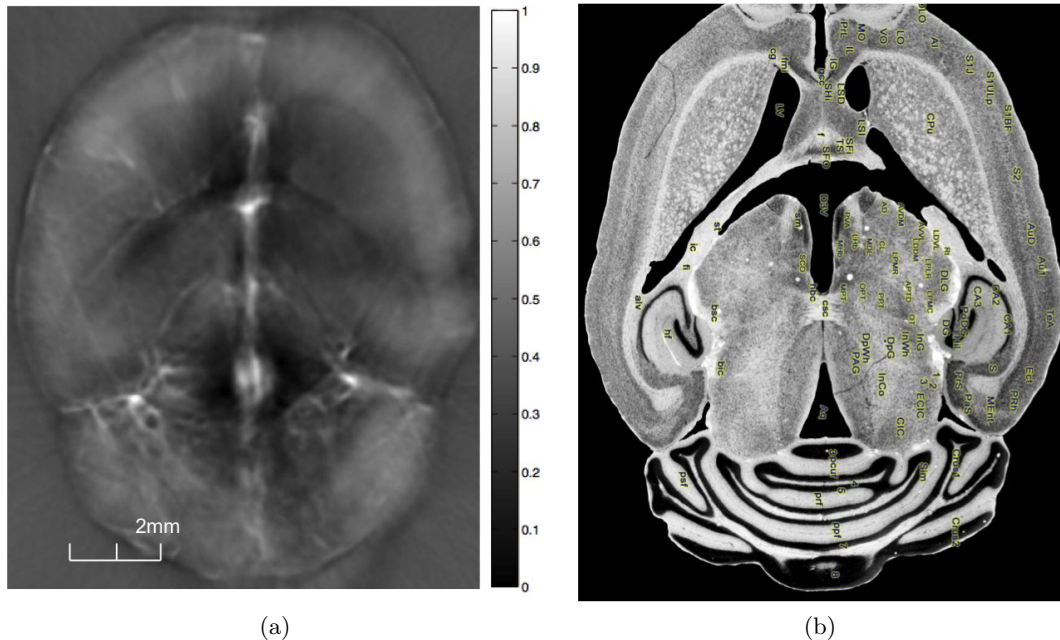


Figure 2. Comparison between the PACT image and the standard mouse brain atlas. (a) the PACT image. Experiment #113012, wavelength 600 nm, depth around 3 mm, field of view 11mm by 13.6 mm. (b) Atlas at 4.4 mm below bregma<sup>[12]</sup>. Note that the color bars used throughout this report are normalized photoacoustic amplitude.

Figure 3.1 shows the corresponding structure matching between a PACT image and the atlas. The red circle is the striatum, which is closely related to cognitive processes such as working memory<sup>[13]</sup>. It is involved in Parkinson's disease and bipolar disorder<sup>[14][15]</sup>. The yellow boundary encloses indicates the left and right ventricles and the dorsal 3rd ventricle, which are important components of the ventricular system in the brain. The ventricular system protects the brain from trauma, and circulates cerebrospinal fluid to the central canal. Ventricle deformation is related to mental diseases such as schizophrenia. The green triangle encloses the cerebral aqueduct, which connects the 3rd ventricle to the 4th ventricle. A block in the cerebral aqueduct will cause problems in cerebrospinal fluid circulation. The brown region encloses the inferior colliculus part in the brain stem, which plays a critical role in the auditory pathway. The blue circles outline the pretectal nucleus (which allows adjusting to acute changes of illumination), the lateral geniculate body (the relay center for the visual pathway) and the thalamus.

Zooming in and adjusting the image contrast, we can see a clear match in the hippocampus, as illustrated in figure 3.1. The hippocampus is extensively studied in both rodents and humans for its role in spatial memory, navigation, and memory-related neural activities. It is closely involved in to aging and memory-involved diseases. We won't go into details about its importance for there is plenty of literature on its functions, mechanisms, and pathology. In figure 3.1, it can be seen that in the PACT image, some details in the hippocampus (indicated by label CA1/2/3, PoDG in 4(b)) can also be differentiated.





Experiment #113012's result shows that *ex vivo* PACT images of a mouse brain can differentiate almost all the important structures at a depth of around 3 mm with high resolution.

### 3.2 6 mm depth

A similar experiment (experiment # 122012) shows a clear artery feature at a depth of 6 mm in figure 5(a). Figure 5(b) is a bottom-up-view photomicrograph of a carbon-ink injected mouse brain, which shows an artery feature called the Circle of Willis<sup>[16]</sup>, which is a significant physiological marker in the brain vessel system. Located at the bottom of the brain, it is in charge of blood supply and circulation for the brain. Note that the reconstruction algorithm used here is the three-dimensional back projection method proposed in Xia *et al.*<sup>[11]</sup>, which cancels out out-of-plane artifacts.

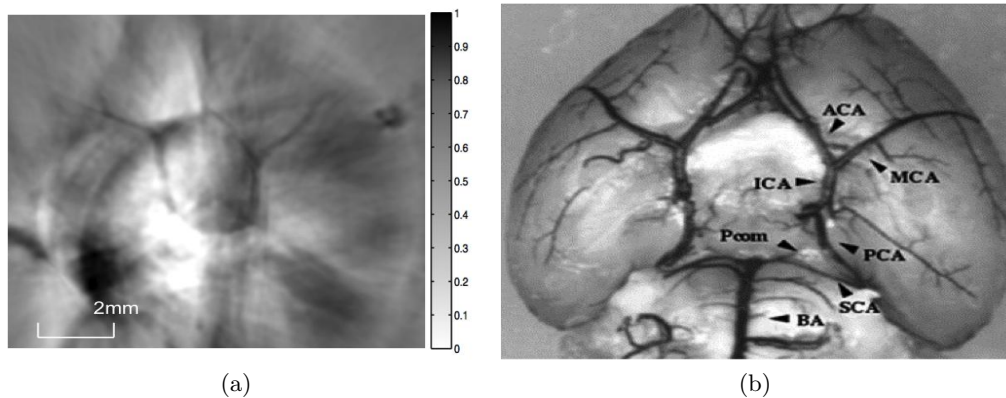


Figure 5. Circle of Willis comparison. 5(a) the PACT image of mouse brain, experiment #122012, wavelength 600 nm, imaging depth 6 mm, field of view: 12.9 mm by 12.4 mm. 5(b) the photomicrograph of Circle of Willis<sup>[16]</sup>. Mouse was injected with carbon ink.

It can be seen that there is a very good match between the PACT image and the photograph, including the cerebral arteries (ACA, MCA and PCA in figure 5(b)), an internal carotid artery (ICA), and a posterior communication artery (PcomA). Seeing features at the bottom of the brain sample means PACT *ex vivo* imaging can penetrate all the way through the brain sample. This experiment demonstrates that PACT can reach a very deep penetration depth with satisfying resolution.

## 4. DISCUSSION

In section 3 we showed PACT images of an *ex vivo* mouse brain to demonstrate the resolution and penetration depth of PACT. The depth we used in section 3 is an approximation of the real depth. We used the distance between the starting slice and the chosen slice as a measurement of depth. Since it is hard to find a landmark on the surface of the brain, we set our starting slice as the depth where the central cortex was nicely focused. Hence, the depth here might be different from that of previous work such as Wang *et al.*<sup>[2]</sup>. Section 2 mentioned that we mainly used blood in the brain as the contrast, so as much as possible blood was preserved in our experiments. We later found that some major components of white matter, gray matter and caudate/putamen can also serve as imaging contrast in PACT brain imaging. Combined with absorption spectrum of these components, this very exciting finding suggests a new way to determine the relative concentration of different brain tissue components in fresh brain, even *in vivo*. This new goal is part of our future work.

## 5. SUMMARY

In this paper, we demonstrated how PACT *ex vivo* mouse brain imaging can reach high resolution (indicated by its ability to separate structures) and deep penetration depth at the same time. The significance of our work lies in the following aspects:

1. Experiment #113012 shows that PACT can reach high resolution at 3 mm. At this depth, different brain structures, such as the striatum and the hippocampus are widely studied in neuroscience and brain pathology. Being able to differentiate and see details of these structures proves PACT's great potential to be used for related research.
2. Experiment #122012 shows that PACT's penetration covers the whole brain while still revealing important structure. This result can serve as an imaging depth benchmark for future study in PAT brain imaging.

In conclusion, our work can serve as a reference for PACT brain imaging in terms of both what can be seen and how deeply PACT can image.

## ACKNOWLEDGMENTS

This work was supported in part by NIH awards DP1 EB016986, R01 CA186567, R01 EB 016963, R01 CA134539, R01 EB 010049, R01 CA157277 and R01 CA 159959.

## References

- [1] Wang, L., [*Photoacoustic imaging and spectroscopy*], vol. 144, CRC (2009).
- [2] Wang, X., Pang, Y., Ku, G., Stoica, G., and Wang, L., "Three-dimensional laser-induced photoacoustic tomography of mouse brain with the skin and skull intact," *Optics letters* **28**(19), 1739–1741 (2003).
- [3] Nasirivanaki, M., Xia, J., Wan, H., Bauer, A. Q., Culver, J. P., and Wang, L. V., "High-resolution photoacoustic tomography of resting-state functional connectivity in the mouse brain," *Proceedings of the National Academy of Sciences* **111**(1), 21–26 (2014).
- [4] Wang, X., Pang, Y., Ku, G., Xie, X., Stoica, G., Wang, L., et al., "Noninvasive laser-induced photoacoustic tomography for structural and functional in vivo imaging of the brain," *Nature biotechnology* **21**(7), 803–806 (2003).
- [5] Wang, Y., Xie, X., Wang, X., Ku, G., Gill, K., O'Neal, D., Stoica, G., and Wang, L., "Photoacoustic tomography of a nanoshell contrast agent in the in vivo rat brain," *Nano Letters* **4**(9), 1689–1692 (2004).
- [6] Li, M., Oh, J., Xie, X., Ku, G., Wang, W., Li, C., Lungu, G., Stoica, G., and Wang, L., "Simultaneous molecular and hypoxia imaging of brain tumors in vivo using spectroscopic photoacoustic tomography," *PROCEEDINGS-IEEE* **96**(3), 481 (2008).
- [7] Yao, J., Xia, J., Maslov, K. I., Nasirivanaki, M., Tsytsarev, V., Demchenko, A. V., and Wang, L. V., "Non-invasive photoacoustic computed tomography of mouse brain metabolism in vivo," *Neuroimage* **64**, 257–266 (2013).
- [8] Spijker, S., "Dissection of rodent brain regions," *Neuroproteomics* **57**, 13–26 (2011).
- [9] Gamelin, J., Maurudis, A., Aguirre, A., Huang, F., Guo, P., Wang, L., and Zhu, Q., "A real-time photoacoustic tomography system for small animals," *Optics express* **17**(13), 10489–10498 (2009).
- [10] Xia, J., Chatni, M. R., Maslov, K., Guo, Z., Wang, K., Anastasio, M., and Wang, L. V., "Whole-body ring-shaped confocal photoacoustic computed tomography of small animals in vivo," *Journal of biomedical optics* **17**(5), 0505061–0505063 (2012).
- [11] Xia, J., Guo, Z., Maslov, K., Aguirre, A., Zhu, Q., Percival, C., and Wang, L., "Three-dimensional photoacoustic tomography based on the focal-line concept," *Journal of biomedical optics* **16**(9), 090505–090505 (2011).
- [12] "The mouse brain library." [http://www.mbl.org/atlas232/atlas232\\_frame.html](http://www.mbl.org/atlas232/atlas232_frame.html).

- [13] Voytek, B. and Knight, R., “Prefrontal cortex and basal ganglia contributions to visual working memory,” *Proceedings of the National Academy of Sciences* **107**(42), 18167–18172 (2010).
- [14] Walker, F., “Huntington’s disease,” *The Lancet* **369**(9557), 218–228 (2007).
- [15] “Scientists pinpoint gene variations linked to higher risk of bipolar disorder.” [http://www.sciencedaily.com/releases/2012/10/121010151422.htm?utm\\_source=feedburner&utm\\_medium=email&utm\\_campaign=Feed%3A+sciencedaily+%28ScienceDaily%3A+Latest+Science+News%29](http://www.sciencedaily.com/releases/2012/10/121010151422.htm?utm_source=feedburner&utm_medium=email&utm_campaign=Feed%3A+sciencedaily+%28ScienceDaily%3A+Latest+Science+News%29).
- [16] Tsuchiya, D., Hong, S., Kayama, T., Panter, S., and Weinstein, P., “Effect of suture size and carotid clip application upon blood flow and infarct volume after permanent and temporary middle cerebral artery occlusion in mice,” *Brain research* **970**(1), 131–139 (2003).